Supplementary Information (SI) for Lab on a Chip.

Vascular Architecture-on-Chip: Engineering complex blood vessels for reproducing physiological and heterogenous hemodynamics and endothelial function

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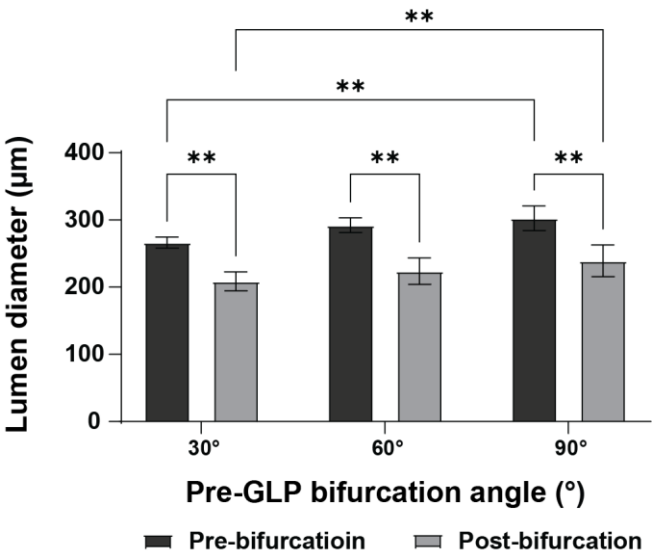
SUPPLEMENTARY INFORMATION

FIGURES



Supplementary Fig. 1 Statistical analysis of pre-bifurcation and post-bifurcation inner diameter. The bifurcated lumen after bifurcation showed significant reduction in the lumen diameter compared to the major vascular lumen before bifurcation for 30°, 60°, and 90° bifurcation angle variations.

METHODS

Supplementary method 1: Gravitational Lumen Patterning:

1. Keep the glutaraldehyde treated devices under vacuum for 1 hour to remove air pockets and moisture from the devices.
2. Bring the GLP reagents out on ice and quick spin the collagen aliquot in a tabletop centrifuge.
3. Add the components sequentially into a sterile micro-centrifuge tube and mix at each step to prepare the collagen mix (5 or 7.5 mg mL^{-1}).
4. Check the pH of the solution, it should be around 7.5.

5. Bend 200 µL pipette tips to nearly 135º about 7 mm into the tip length.
6. Pushed the bent tips into the outlet ports of the channel till tips reached the top of the microfluidic channels.
7. For bifurcated channels, use two curved tips considering the two ports at the ends of the bifurcated branches as outlet ports.
8. Inject 20 µL per channel through inlet and remove the tip.
9. Leave the collagen filled device flat on surface for 15 seconds for equilibration then rotate the device vertically.
10. Immediately add 40 µL of PBS through the inlet using a bent tip while the tip is loosely connected to the pipette.
11. Incubate the vertically oriented device for 7 mins in 5% CO_2 37ºC humidified incubator.
    **Vertical orientation allows gravitational force to act along the vessel's axial direction and prevents buoyant effect which pushes the lumen closer to the upper surface of the channel.*
12. Retrieve the device from the incubator and remove the tips using a rotating motion.
13. Wash the channels with 200 µL PBS (or media) and incubate for at least an hour in 5% CO_2 37ºC humidified incubator.

MOVIES

Supplementary Movie 1 3D confocal micrograph of cylindrical vessel-chip lumen with confluent monolayer of human umbilical vein endothelial cells (HUVECs). The cells were stained for VE-cadherin (green) and nuclei (blue) after 24 hours of cell culture. The cells show alignment along the direction of flow and conserved barrier function.

Supplementary Movie 2 3D confocal micrograph of aneurysm-on-chip lumen with confluent monolayer of HUVECs. The cells were stained for VE-cadherin (green) and nuclei (blue) after 24 hours of cell culture. The cells in cylindrical region of the vessel exhibit alignment along the flow direction, but the cells in aneurysm region shows more random orientation.

Supplementary Movie 3 3D confocal micrograph of stenosis-on-chip lumen with confluent monolayer of HUVECs. The cells were VE-cadherin (green) and nuclei (blue) after 24 hours of cell culture.

Supplementary Movie 4 3D confocal micrograph of bifurcation-on-chip lumen with confluent monolayer of HUVECs. The cells were VE-cadherin (green) and nuclei (blue) after 24 hours of cell culture.

Supplementary Movie 5 3D confocal micrograph of tortuous vessel-chip lumen with confluent monolayer of HUVECs. The cells were VE-cadherin (green) and nuclei (blue) after 24 hours of cell culture.

Supplementary Movie 6 Red blood cell (RBC) perfusion through cylindrical vessel-chip. The lumen integrity was conserved.

Supplementary Movie 7 RBC perfusion through aneurysm-on-chip. RBCs in the extremities of the aneurysm move slower than those in the middle of the aneurysm. The lumen integrity was conserved.

Supplementary Movie 8 RBC perfusion through stenosis-on-chip. RBCs move faster through the stenosis region compared to the rest of the vessel without any constriction. The lumen integrity was conserved.

Supplementary Movie 9 RBC perfusion through bifurcation-on-chip. RBCs divide evenly into the two post-bifurcation sections. The lumen integrity was conserved.

Supplementary Movie 10 RBC perfusion through tortuous vessel-chip. RBCs in the extremities of the curves move slower than those in the straight region of the vessel. The lumen integrity was conserved.